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Short communication

Estimation of thermodynamic acidity constants of some penicillinase-resistant penicillins

Ebru Çubuk Demiralay*, Zehra Üstün, Y. Doğan Daldal

Department of Chemistry, Faculty of Science and Literature, Süleyman Demirel University, 32260 Isparta, Turkey

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ABSTRACT

In this work, thermodynamic acidity constants ($^{s}_{s}pK_a$) of methicillin, oxacillin, nafcillin, cloxacilin, dicloxacillin were determined with reverse phase liquid chromatographic method (RPLC) by taking into account the effect of the activity coefficients in hydro-organic water–acetonitrile binary mixtures. From these values, thermodynamic aqueous acidity constants of these drugs were calculated by different approaches. The linear relationships established between retention factors of the species and the polarity parameter of the mobile phase (E_T^N) was proved to predict accurately retention in LC as a function of the acetonitrile content (38%, 40% and 42%, v/v).

1. Introduction

Penicillins have been widely used antimicrobial drugs for more than 80 years and still are considered as one of the most important groups of antibiotics. They are derived from the penicillium mold and belong to the β-lactam group of antibiotics used in the treatment of bacterial infections caused by susceptible, usually gram positive and gram negative organisms [1,2]. The basic structure of penicillin is a thiazolidine ring attached to a β-lactam ring. Five members of this class of antibiotics were determined in the present study: oxacillin, methicillin, nafcillin, dicloxacillin and cloxacillin. Methicillin was the first of the series of drugs known as penicillinase resistant penicillins [1,2].

Acidity constants (pK_a) are essential physicochemical parameters to indicate the extent of ionization of molecules in solution at different pH values. The knowledge of these parameters is crucial importance to estimate chemical absorption, distribution, metabolism, excretion, formulation development. Moreover, acidity constant values established for organic solvent–water binary mixture systems can be extremely useful for prediction of retention and optimization of chromatographic separations of compounds [3,4].

Several methodologies have been proposed for the determination of acidity constants. High performance liquid chromatography (HPLC), capillary electrophoresis (CE), capillary isotachophoresis, potentiometry, UV–vis spectrometry and theoretical calculations of pK_a values are frequently used for the determination of acidity constant value of ionizable analytes [5–10]. Among these techniques, estimation of acidity constant of drugs can be analyzed by HPLC technique because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation. The determination of acidity constant value by liquid chromatographic method is based on a relationship between the retention factors and the pH values of the mobile phase. Different authors have realized that better results are obtained when the pH in the mobile phase is taken into consideration instead of aqueous pH of the buffer [3,6,11].

Analysis of the change in retention time of the analyte versus the change in pH of the mobile phase is give an indirect measure of the acidity constant. The theory for studying the pH dependence of chromatographic retention for ionizable compounds in LC was proposed by Horvath et al. [12]. The expression:

$$k = \frac{k_{HA} + k_{A^-}(K_a/a_{H_m^+}\gamma_{A_m^-})}{1 + (K_a/a_{H_m^+}\gamma_{A_m^-})} \quad (1)$$

represents the variation of the retention factor for a weak monoprotic acid (HA) with the hydrogen ion activity in the mobile phase, ($a_{H_m^+}$). HA and A^- represent undissociated acid and fully dissociated acid, respectively. The acidity constant in the acetonitrile–water

* Corresponding author. Tel.: +90 246 211416; fax: +90 246 2371106.
E-mail addresses: ebrucubuk@sdu.edu.tr, ebrucubuk@mynet.com.tr (E.Ç. Demiralay).

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mixture used as mobile phase is represented by K_a. $\gamma_{A_m^-}$ is the activity coefficient of the dissociated acid in the mobile phase that can be calculated by the classical Debye–Hückel equation [13]. The observed retention factor (k) is an average of the retention factors of the acid (k_{HA}) and basic forms (k_{A^-}). It is known that these factors are very useful to explain the differences between the affinity of the ionized and neutral form to the stationary phase.

Linear plots of $\log k$ versus the φ are also satisfactory for most solutes for narrow ranges of organic solvent. However, Johnson et al. [14] demonstrated linear relationships in a wide range of organic solvents between the solute $\log k$ and E_T^N [15]. A widely employed measure of solvent polarity is Dimroth and Reichardt's $E_T(30)$ [16]. This polarity index is often used in the normalized dimensionless form, E_T^N. E_T^N polarity parameter have been used to predict the chromatographic behavior of ionizable compounds.

Variation of the retention factor of neutral form of an acid (k_{HA}) and the retention factor of anionic form of an acid (k_{A^-}) with percentage of acetonitrile in the mobile phase is represented by the normalized Dimroth and Reichardt polarity parameter (Eqs. (2) and (3)).

$$\log k_{HA} = C_{HA} + e_{HA}E_T^N \quad (2)$$

$$\log k_{A^-} = C_{A^-} + e_{A^-}E_T^N \quad (3)$$

Substituting Eqs. (2) and (3) into Eq. (1) the theoretical expression describing the dependence of the retention factor for acidic solutes as a combined function of pH and E_T^N may be expressed as follows:

$$k = \frac{10^{(C_{HA}+e_{HA}E_T^N)} + 10^{(C_{A^-}+e_{A^-}E_T^N)}(K_a/a_{H_m^+}\gamma_{A_m^-})}{1 + (K_a/a_{H_m^+}\gamma_{A_m^-})} \quad (4)$$

where $a_{H_m^+}$ is the thermodynamic activity of the indicated species in the binary mixture. C_{HA} and C_{A^-} are intercept values of the neutral and anionic species, respectively. Similarly, e_{HA} and e_{A^-} are slope values of these species [17].

The thermodynamic acidity constant values of methicillin, oxacillin, nafcillin, cloxacillin, dicloxacillin are either not known accurately or not available at all. In this study, acidity constant values of these substances in different percentages of acetonitrile–water binary mixtures (38%, 40% and 42%, v/v) were determined by using the variation of retention factors of these drug candidates as a function of the mobile phase pH. The main objective of the present study was to determine thermodynamic aqueous acidity constant values ($^w_wpK_a$) of these compounds by means of Yasuda–Shedlovsky equation [18] and linear relationship between the mole fraction of acetonitrile and the $^s_spK_a$ values. Yasuda–Shedlovsky equation (Eq. (5)) was applied to estimate the thermodynamic aqueous acidity constant.

$$pK_a + \log[H_2O] = a_\varepsilon \varepsilon^{-1} + b_\varepsilon \quad (5)$$

where $\log[H_2O]$ is the molar water concentration of the given solvent mixture, ε is the dielectric constant of the mixture and a and b are the slope and intercept, respectively.

2. Experimental

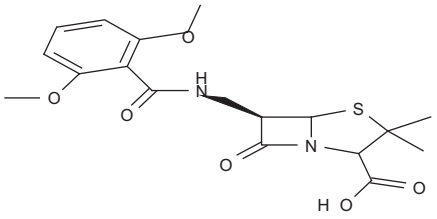
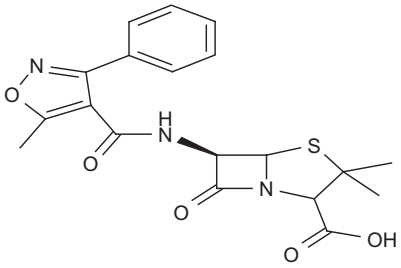
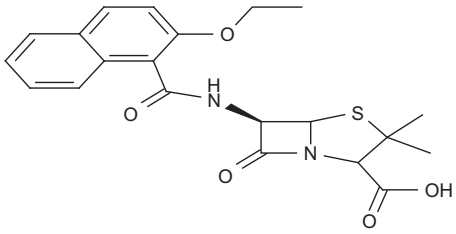
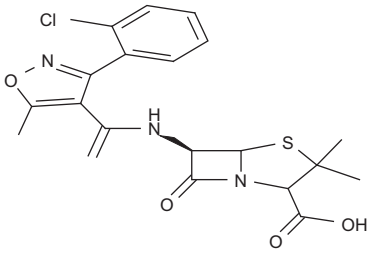
2.1. Materials

Analyte drugs (purity ≥ 99%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) and sodium hydroxide (used in pH adjustment) were also obtained from Merck (Darmstadt, Germany). Ortho-phosphoric acid was analytical grade and obtained from Riedel-de Haen (Hannover, Germany).

2.2. Instrumentation

The chromatographic experiments were carried out on a Shimadzu HPLC system (Shimadzu Technologies, Kyoto, Japan). The Shimadzu HPLC system was operated using a LC-10AD VP pump, a SPD-10AV VP UV Visible detector, a CTO-10AC VP column oven and a DGU-14A degasser system. Synergy Fusion-RP C18 analytical column (150 mm × 4.6 mm I.D., 4 μm, 80 Å) was used as stationary phase (Phenomenex, Torrance, USA). In order to measure the pH values of the eluents, a Mettler Toledo MA 235 pH/ion analyser (Schwerzenbach, Switzerland) was chosen. For the standardization of potentiometric system according to the IUPAC rules [19], potassium hydrogen phthalate (0.05 mol/kg) was used. The activity coefficients, γ, were calculated using the Debye–Huckel equation taking into account the ionic strength, I, of the mobile phases [13].

Table 1
Chemical structures of methicillin, oxacillin, nafcillin, cloxacillin, dicloxacillin.

Compounds	Structure
Methicillin	
Oxacillin	
Nafcillin	
Cloxacillin	
Dicloxacillin